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Expression of the Melanoma Antigen-Encoding Gene in Human Lung Cancer

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Background and Objectives: We surveyed expression of melanoma antigen-encoding genes in lung cancer because of promising implications for immunotherapy.

Methods: We studied 57 human lung carcinoma specimens using the reverse transcripion-polymerase chain reaction (RT-PCR).

Results: In the samples, the expression of melanoma antigen-encoding genes 1, 2, and 3 was observed in 9/43 (20.9%), 13/43 (30.2%), and 22/48 (45.8%), respectively. In 28 cases in which all three messenger RNAs were sought, 18 (64.3%) showed expression of at least one gene, 10 (35.7%) showed expression of two or three genes, and 10 (35.7%) were negative for all three genes. In a clinicopathologic analysis, melanoma antigen-encoding genes 1 and 3 were frequently expressed in squamous cell carcinomas ($P = 0.0543$) and in cases with regional lymph node metastasis ($P = 0.0572$), respectively.

Conclusions: The high incidence of melanoma antigen-encoding gene expression in lung cancer indicates the possibility of a future specific immunotherapy for this disease.

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KEY WORDS: tumor rejection antigen; MAGE gene; lung cancer; immunotherapy

INTRODUCTION

Recently, melanoma-associated antigens such as the products of melanoma antigen-encoding genes (MAGE) [1], tyrosinase [2], MART-1/Melan-A [3], and Pmel 17/gp100 [4] have been identified. These antigens are often shared among patients with the same histopathology [5,6]. The MAGE genes are most widely expressed in neoplasms such as breast cancer [7], laryngeal tumors [8], sarcoma [9], renal cell carcinoma [9], colon cancer [9], stomach cancer [10], esophageal carcinoma [11], and glioblastoma [12]. However, MAGE genes are not expressed in normal tissues, with the exception of testis [13], placenta [14], and skin during wound healing [15].

The MAGE-1 and MAGE-3 are known to encode major histocompatibility complex (MHC)-restricted antigens, which can be recognized by melanoma-specific cytotoxic T lymphocytes (CTL) [5,6]. Recently, Boon and colleagues began a clinical trial vaccinating patients with melanoma using an HLA-A1-restricted peptide encoded by MAGE-3. They reported some tumor regression after vaccination [16]. In the current study, we investigated the expression of MAGE-1, -2, and -3 messenger RNA in

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clinical lung cancer in order to examine whether specific immunotherapy using MAGE gene products is applicable.

MATERIALS AND METHODS

Patients

Surgical specimens were obtained from a total of 57 patients with lung cancer who underwent a surgical resection and mediastinal lymph node dissection at the Department of Surgery II, University of Occupational and Environmental Health, Japan. All tumor samples were cut from the specimen and frozen at −70°C immediately after resection. Of the 57 patients, the gene expression of MAGE-1, MAGE-2, and MAGE-3 was analyzed in 43, 43, and 48 patients, respectively. The pathologic stage for each tumor was determined according to the TNM classification [17]. The histologic diagnosis of these tumors was based on the World Health Organization criteria [18]. All data, including sex, age, TNM classification, and relevant clinical information, were obtained from the clinical and pathologic records. All tumors, with or without expression of MAGE genes, were then compared.

Assay for the Expression of MAGE Genes

Total RNA was isolated by acid guanidium-phenol-chloroform and was treated with DNase (Promega, Madison, WI). cDNA was synthesized from 2.5 mg of total RNA as described [10,19]. The presence of MAGE-1, -2, and -3 cDNA in the reverse transcription products was detected by polymerase chain reaction (PCR) amplification in separate reactions using oligonucleotide primers located in different exons of the MAGE genes [19]. The sequences of oligonucleotide primers for MAGE-1, -2, and -3 have been reported previously [5,7,18]. PCR was performed for 35 cycles (1 min at 94°C, 1.5 min at 67°C, and 1 min at 72°C for MAGE-1; and 1 min at 94°C, 1.5 min at 60°C, and 1 min at 72°C for MAGE-2 and MAGE-3). In this study, 1 μl of each PCR product was size-fractionated on a 2% agarose gel and then stained with ethidium bromide. To ensure that the RNA was not degraded, a PCR assay with primers specific for b-actin was performed in each case as a positive control [20].

Statistical Analysis

Statistical analysis was performed using the Fisher's exact test.

RESULTS

Messenger RNA for MAGE-1, MAGE-2, and MAGE-3 was amplified by RT-PCR and detected as bands of 421, 236, and 725 bases, respectively. β-actin (approximate length, 800 bases) was also detected in all cases as a control (Fig. 1). The expression of MAGE-1, MAGE-2, and MAGE-3 was observed in 9 of 43 patients (20.9%), 13 of 43 (30.2%), and 22 of 48 (45.8%) patients, respectively.

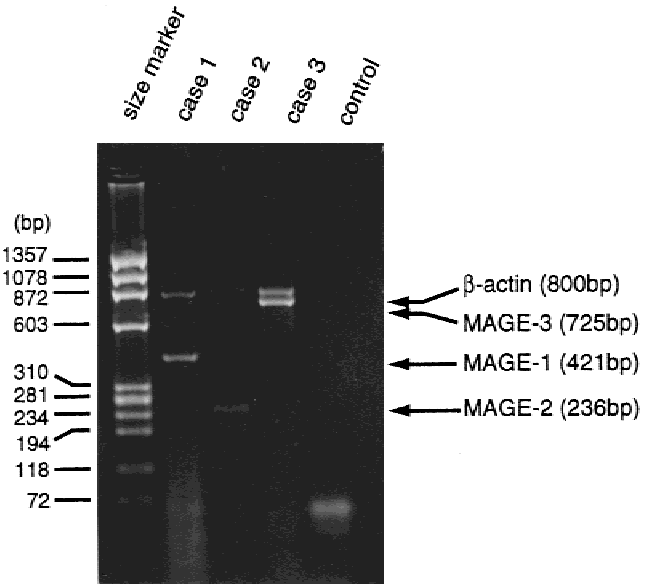

Fig. 1. Amplified MAGE-1, MAGE-2, and MAGE-3 genes by reverse transcription-polymerase chain reaction in three representative cases.

TABLE I. Expression Pattern of MAGE Genes in 28 Patients With Lung Cancer Who Were Tested for the Expression of MAGE-1, -2, and -3 Simultaneously

Expressed MAGE gene(s)	No. in positive cases	Proportion (%)
MAGE-1 only	2	7.1
MAGE-2 only	3	10.7
MAGE-3 only	3	10.7
MAGE-1 and MAGE-2	0	0
MAGE-1 and MAGE-3	3	10.7
MAGE-2 and MAGE-3	5	17.9
All three genes	2	7.1
None	10	35.7
Total	28	100

The pattern of MAGE-1, MAGE-2, and MAGE-3 gene expression in the lung cancer patients (n = 28), in whom all three genes were studied, is shown in Table I. At least one gene was expressed in 18 cases (64.3%), MAGE-1 and MAGE-3 in 3 cases (10.7%), MAGE-2 and MAGE-3 in 5 cases (17.9%), and all three genes in 2 cases (7.1%). Ten specimens (35.7%) were negative for all three genes.

As to MAGE-1, the proportion of positive cases was 37.5% (6/16 cases tested) in squamous cell carcinoma, and 12.0% (3/25) in adenocarcinoma, as to MAGE-2, that was 33.3% (6/18) in squamous cell carcinoma, and 33.3% (7/21) in adenocarcinoma, and as to MAGE-3, that was 61.1% (11/18) in squamous cell carcinoma, and 37.0% (10/27) in adenocarcinoma (Table II). A significant correlation between MAGE gene expression and clinicopathologic factors was not shown in this study (Table II). The frequency of MAGE-1 gene expression,

TABLE II. Clinicopathologic Data of All Cases of Primary Lung Cancer

	MAGE-1 (n = 43)		MAGE-2 (n = 43)		MAGE-3 (n = 48)	
	+	−	+	−	+	−
No. of patients	9 (20.9)[a]	34 (79.1)	13 (30.2)	30 (69.8)	22 (45.8)	26 (54.2)
Age (mean; year)	71.6	64.2	62.5	67.3	65.7	65.4
Sex						
Male	7	24	10	21	17	18
Female	2	10	3	9	5	8
Histology						
Sq	6	10	6	12	11	7
Ad	3	22	7	14	10	17
Ad-Sq	0	1	0	2	0	1
Sm	0	1	0	1	0	1
La	0	0	0	1	1	0
Stage						
I	2	11	5	7	3	8
II	1	0	1	2	1	2
IIIA	4	14	4	16	11	12
IIIB	2	4	1	2	3	2
IV	0	5	2	3	4	2
Differentiation						
Well	0	3	0	2	0	1
Moderate	7	20	11	19	15	17
Poorly	2	9	2	4	5	6
Lymph node metastasis						
Absent	2	17	7	9	4	11
Present	7	15	6	18	17	13

[a]Proporation (%) of (+) or (−) in the total cases tested.
Ad, adenocarcinoma; Sq, squamous cell carcinoma; Ad-Sq, adenosquamous cell carcinoma; Sm, small cell carcinoma; La, large cell carcinoma.

however, was higher in squamous cell carcinomas than in adenocarcinomas ($P = 0.0543$). In addition, MAGE-3 gene expression was higher in cases with regional lymph node metastasis than in those without metastases ($P = 0.0572$). The expression of MAGE genes was not observed in one patient with small cell carcinoma, and in two patients with adenosquamous cell carcinoma.

DISCUSSION

In non-small cell lung cancer, the expression of MAGE genes were already reported by Weynants et al. [19]. In their study, the expression of MAGE-1, MAGE-2 and MAGE-3 is 35.0%, 35.0%, and 30.4%. In our study, the expression of MAGE-1 was lower (21%), and that of MAGE-3 was higher (46%), as compared with the above report. This difference may be due to differences in racial characteristics of Caucasians in the study conducted by Weynants et al. and Japanese individuals in the present study, since a tumor-rejection antigen encoded by MAGE genes was recognized by CTL in a MHC-restricted manner, and the frequency of each MHC haplotype is unique to each race. For instance, approximately 50% of Caucasians express HLA-A2 [21], whereas approximately 20–40% of Japanese express HLA-A2 [22]. In particular, if a MAGE-3 gene product derived antigenic peptide (271–279)[6] is HLA-A2 restricted, MAGE-3-positive cancer cells would be recognized and killed by CTL.

The MAGE family of genes is known to be expressed in many histologic types of malignant tumors. Lung cancer may be subdivided into various histologic types, including squamous cell carcinoma, adenocarcinoma, large cell carcinoma, and small cell carcinoma. In this study, two major types of lung cancer, adenocarcinoma and squamous cell carcinoma, expressed MAGE genes, but not in patients with small cell carcinoma or adenosquamous cell carcinoma. Follow-up evaluation of the patients in this study is too short to study for the relationship between the expression of MAGE genes and patient survival.

In addition to MAGE genes, tyrosinase, MART-1, and Pmel 17 were isolated [1–4]. The MAGE-1- and -3-derived antigens were first reported to be recognized only by CTL in an HLA A1- and Cw16-restricted fashion [23,24]. Recently, however, it was reported that another peptide encoded by MAGE-3 gene (nonomer oligopeptide; FLWGPRALV) is presented by HLA-A2 and recognized by CTL [6]. From this information, Boon and

colleagues have started clinical vaccine trial using this peptide because high frequency of HLA-A2 in Caucasians. In our unpublished data, 42.1% (16 of 38 patients) of patients with lung cancer have HLA-A2. Frequent expression of MAGE-3 gene in lung cancer, especially in squamous cell carcinoma, and frequent expression of HLA-A2 in Japanese people as well as Caucasians prompt us to further investigate the possibility of specific immunotherapy against lung cancer using a peptide encoded by MAGE-3 with a binding motif to HLA-A2.

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